Early weaning alters the acute-phase reaction to an endotoxin challenge in beef calves[1]

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ABSTRACT: Previous research indicates that early weaning before shipment can reduce transportation-induced increases in acute-phase proteins (APP) and can increase feedlot performance in beef calves. These data suggest that the combination of weaning and transport stress may compromise the immune system of calves, thus hindering subsequent performance and health. Therefore, our objective was to determine if the innate immune response of early weaned calves (EW; 80 d of age) differed from normal-weaned calves (NW; 250 d of age) in response to an endotoxin challenge. Eighteen Brahman × Angus calves (8 and 10 EW and NW, respectively; 233 ± 5 kg of BW) were used. Calves were maintained on pasture with supplement and then moved into individual pens for 1 wk of acclimation before the start of the study. Calves were fitted with an indwelling jugular catheter 1 d before LPS challenge (0 h; 1.0 μg/kg of BW, intravenously). Blood samples were collected at 30-min intervals from −2 to 8 h. Serum samples were stored at −80°C until analyzed for cortisol, tumor necrosis factor-α (TNF), IL-1 β, IL-6, interferon-γ (IFN), ceruloplasmin, and haptoglobin. Whereas LPS increased serum cortisol ($P \leq 0.001$), no weaning age effect ($P \geq 0.15$) was observed. A weaning age × time interaction ($P \leq 0.04$) was observed for TNF, IL-1, IL-6, and ceruloplasmin such that concentrations of these indices were greater in the NW compared with EW calves. For haptoglobin, a weaning age effect ($P \leq 0.03$) was observed with NW calves having greater average haptoglobin concentrations compared with EW calves. Interestingly, the weaning age × time interaction ($P \leq 0.001$) for IFN revealed greater IFN in EW compared with NW calves. Based upon these data, the innate immune system of EW calves appears to be more competent in responding to immune challenge compared with that of NW calves. Additionally, the differential IFN response indicates that the immune system of EW calves may be more effective at recognizing and eliminating endotoxin. These data suggest that an altered innate immune system may be one of the factors responsible for the improved feedlot performance previously reported in EW calves.

Key words: acute-phase response, calf, cattle, weaning

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INTRODUCTION

The ability of an animal to readily respond and adapt to stress stimuli likely influences the potential for a disease outcome. Many stressors associated with the production of beef are the result of normal management procedures. One such event is the occurrence of weaning. In beef cattle, this is typically an artificial, permanent separation of the cow and calf, which results in decreased time spent eating and increased walking and vocalization (Veissier and LeNeindre, 1989; Price et al., 2003). In addition to these behavioral responses, blood concentrations of catecholamines are increased in calves after weaning (Lefcourt and Elsasser, 1995; Hickey et al., 2003). This stress response may also elicit the onset of the proinflammatory response. This immunological reaction involves a complex set of reactions including the release of multiple soluble mediators impacting the metabolic response of the host to inflammation (Baumann and Gauldie, 1994). An important group of soluble products of the proinflammatory response include the acute-phase proteins (**APP**), which are increased after weaning and transportation in beef calves (Arthington et al., 2003). Concentrations of APP have been shown to be reduced in transport-stressed early weaned (**EW**) calves compared with normal-weaned (**NW**) contempo-

[1]Mention of trade names or proprietary products does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

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raries (Arthington et al., 2005, 2008), but the reasons for this response are currently unknown. Therefore, the objective of the current study was to determine if the selected responses of innate immunity in EW calves (80 d of age) and NW calves (250 d of age) in response to an intravenous (**i.v.**) endotoxin challenge.

MATERIALS AND METHODS

This experiment was conducted at the University of Florida, Range Cattle Research and Education Center, Ona. All procedures were reviewed and approved by the University of Florida, Institute of Food and Agricultural Sciences, Animal Research Committee.

Animals and Sample Collection

Brahman × Angus calves (n = 18; 233 ± 5 kg of BW) were used in the current study. Treatments were derived from 2 weaning ages consisting of EW (n = 8; weaned at 80 d of age) and NW (n = 10; weaned at 245 d of age) calves. Within weaning age treatment, there were 5 and 3, and 5 and 5 steers and heifers for EW and NW groups, respectively. Early weaned calves were maintained on ryegrass (*Lolium multiflorum*) pastures (January to May) and limpograss pastures (*Hemarthria altissima*) from May to the start of the study in August. Calves were provided supplement (80:20 blend of soybean hulls and cottonseed meal) daily at 1.0% of BW. Normal-weaned calves were weaned from their dams at 45 d before the beginning of the study and maintained on limpograss pastures as a group along with the EW calves. All calves, pre- and postweaning, were provided free-choice access to a salt-based trace mineral mix that contained 12.0% Ca, 9.0% P, 9.0% Na, 0.30% Zn, 0.15% Cu, 0.05% Mn, 0.02% I, 0.005% Co, and 0.004% Se; as-fed basis. One week before the endotoxin challenge and serial blood collection, all calves were housed in their respective individual study pens (4 × 6 m) for acclimation. During this time, calves were provided free choice access to ground stargrass (*Cynodon nlemfuensis*) hay (10.1% CP and 54% TDN) and the same supplement as provided on pasture. One day before administration of lipopolysaccharide (**LPS**; *Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO), all calves were fitted with an indwelling jugular vein catheter for serial blood collection. Catheters consisted of approximately 150 mm of polytetrafluoroethylene tubing (6417–41 18TW, Cole-Palmer; o.d. = 1.66 mm) that was inserted into a jugular vein using a 14-gauge × 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). The catheter was maintained in place using tag cement and a 5.1-cm-wide porous surgical tape. An extension consisting of sterile plastic tubing (Tygon S-50 HL; VWR Scientific, West Chester, PA; i.d. = 1.59 mm; o.d. = 3.18 mm) was attached to the catheter for collection of blood samples with minimal animal disturbance. Between blood samples, all catheters were flushed with 5 mL of saline (0.9% wt/vol NaCl) followed by 5 mL of heparinized saline (1 mL of heparin 10,000 IU/mL in 500 mL of saline) to replace fluid volume and to maintain catheter patency. Two 10-mL blood samples, one in a serum tube and one in an EDTA-coated tube, were collected at 30-min intervals from −2 to 8 h relative to the LPS challenge (time 0). Immediately after sample collection at time 0, all calves received an i.v. bolus dose of LPS (1.0 μg/kg of BW) via the jugular catheter followed by 5 mL of saline and 5 mL of heparinized saline. Blood samples were placed on ice for <1 h before centrifugation at 2,400 × *g* for 30 min at 4°C. Serum and plasma were harvested and stored at −80°C until analysis.

Immune and Endocrine Analyses

All serum analyses were assayed in duplicate or triplicate. Serum cortisol concentrations were determined by radioimmunoassay (Coat-A-Count, DPC, Los Angeles, CA) per the manufacturer's protocol in a single assay with a detection limit of 2 ng/mL and less than 5% intraassay CV. Serum concentrations of the proinflammatory cytokines, tumor necrosis factor-α (**TNF**), IL1-β, IL-6, and interferon-γ (**IFN**) were assayed per the manufacturer's protocol using custom-developed multiplex ELISA validated for bovine cytokines (SearchLight, Pierce Biotechnology Inc., Rockford, IL) with detection ranges of 9.8 to 2,500, 7.8 to 2,000, 3.9 to 1,000, and 2.0 to 500 pg/mL for TNF, IL-1, IL-6, and IFN, respectively. For all cytokines, the intraassay CV was less than 10%, and the interassay CV was less than 15%.

Plasma haptoglobin concentrations were determined in duplicate samples by measuring haptoglobin/hemoglobin complexing by the estimation of differences in peroxidase activity (Makimura and Suzuki, 1982). Results are expressed as arbitrary units resulting from the absorption reading × 100 at 450 nm. For samples with an absorption reading ≤0.010, the intraassay CV of duplicate samples was controlled to values ≤20%, and samples with an absorption reading ≥0.010, the intraassay CV of duplicate samples was controlled to values ≤10%. Plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures described by Demetriou et al. (1974). The intraassay CV of duplicate samples was controlled to values ≤10%. Ceruloplasmin concentrations were expressed as milligrams per deciliter as described by King (1965). Interassay variation of both APP assays was controlled by CV limits ≤10%, as a result of a control sample analyzed in duplicate within each individual assay run. When the interassay CV exceeded 10%, all samples contained in the individual run with the control sample exceeding the average by the greatest were reanalyzed. This step was repeated until the results of standard pools for all runs resulted in a CV ≤10%.

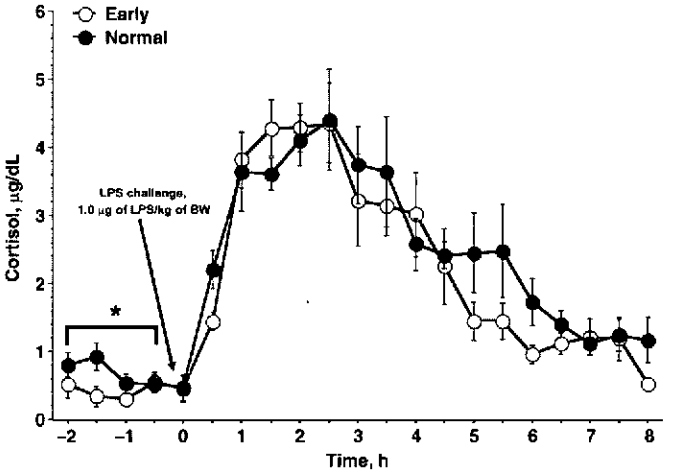

Figure 1. Serum concentrations of cortisol in early weaned (EW; n = 8) and normal-weaned (NW; n = 10) Brahman × Angus calves following an intravenous bolus dose of lipopolysaccharide (LPS; 1.0 µg/kg of BW) via jugular catheter immediately after sample collection at time 0. *During the prechallenge period (−2 to −0.5 h), serum concentrations of cortisol were greater ($P < 0.05$) in the NW vs. EW calves. There was no overall effect of weaning age ($P > 0.15$) or a weaning age × time interaction ($P > 0.81$) on serum concentrations of cortisol. However, there was an overall time effect ($P < 0.0001$) such that serum concentrations of cortisol increased dramatically in both groups after the LPS challenge.

Statistical Analyses

All data were subjected to ANOVA specific for repeated measures using StatView (SAS Inst. Inc., Cary, NC). Sources of variation included weaning treatment, calf sex, time, and their interactions. Calf sex had no significant impact on the main effects or interactions of the cytokines and APP examined in this study; therefore, it was removed from the models for the analyses of these variables. Specific treatment comparisons were made using Fisher's protected least significant difference with comparisons of $P < 0.05$ considered significant. Specific time point comparisons between treatment groups were conducted using an unpaired *t*-test. Specific time point comparisons within a treatment group were conducted using a paired *t*-test.

RESULTS

BW

All calves were weighed 1 d before the LPS challenge to obtain BW for calculating the bolus dose of LPS that would be administered the following day. This initial BW tended ($P < 0.09$) to be greater for EW (242 ± 6.8 kg) compared with NW calves (225 ± 6.3 kg), and within NW calves, heifers were heavier ($P < 0.05$) than their steer cohorts (238 ± 8.9 vs. 214 ± 5.6 kg).

Serum Cortisol

Serum concentrations of cortisol were greater ($P < 0.05$) during the prechallenge period (−2 to −0.5 h) in the NW calves (0.71 + 0.09 µg/dL) compared with EW calves (0.44 + 0.07 µg/dL; Figure 1). However, there was no difference ($P > 0.95$) in serum concentrations of cortisol between the weaning groups immediately before the LPS challenge (i.e., time 0). There was no overall effect of age at weaning ($P > 0.15$) or a weaning age × time interaction ($P > 0.81$) on serum concentrations of cortisol; however, there was an overall time effect ($P < 0.0001$) such that serum concentrations of cortisol increased rapidly in both groups after the LPS challenge. By 0.5 h post-LPS administration, serum concentrations of cortisol had increased ($P < 0.0001$) above prechallenge (mean −2 to −0.5 h) concentrations in both weaning groups and continued to increase until 2.5 h post-LPS challenge. Although there was no weaning age × calf sex × time interaction ($P = 0.97$), heifer calves tended ($P < 0.06$) to have greater peak cortisol concentrations at 2.5 and 3 h post-LPS challenge compared with steer cohorts. Serum concentrations of cortisol gradually began to decline in both weaning groups after the 2.5 h post-LPS sample.

Serum Cytokines

A weaning age × time interaction ($P < 0.0001$) was observed for serum TNF such that serum concentrations of TNF were greater in the NW calves compared with EW calves after the LPS challenge (Figure 2A). Serum concentrations of TNF increased ($P < 0.05$) dramatically in both weaning groups by 1 h post-LPS administration. Peak serum concentrations of TNF occurred at 1.5 h post-LPS challenge and were approximately 2.6-fold greater ($P < 0.001$) in NW (7,442 ± 648.7 pg/mL) calves compared with EW calves (2,901 ± 526 pg/mL). After 1.5 h post-LPS, serum concentrations of TNF declined rapidly in both weaning groups.

For serum concentrations of IL-1, a weaning treatment × time interaction ($P < 0.0001$) was observed such that serum concentration of IL-1 was greater in the NW calves compared with EW calves after the LPS challenge (Figure 2B). Peak serum concentrations of IL-1 occurred at 4.5 h (32 + 5.1 pg/mL) and 5 h (19 + 3.0 pg/mL) post-LPS challenge in the NW and EW calves, respectively. By 8 h post-LPS administration, serum concentrations of IL-1 were not different ($P > 0.14$) from prechallenge concentrations of IL-1 in either weaning group.

Serum concentrations of IL-6 were similar to those for TNF and IL-1 in that a weaning treatment × time interaction ($P < 0.0001$) was observed such that serum concentrations of IL-6 were greater in the NW calves compared with EW calves after the LPS challenge (Figure 2C). The timing of peak serum concentrations of IL-6 were more closely associated with peak IL-1 β concentrations than peak TNF concentrations, occurring at 4.5 h (10,455 ± 1,888.3 pg/mL) and 4 h (6,782 ± 853.5 pg/mL) post-LPS challenge in the NW and EW calves, respectively. However, unlike IL-1, serum concentrations of IL-6 did not return to prechallenge concentrations within the 8 h post-LPS administration

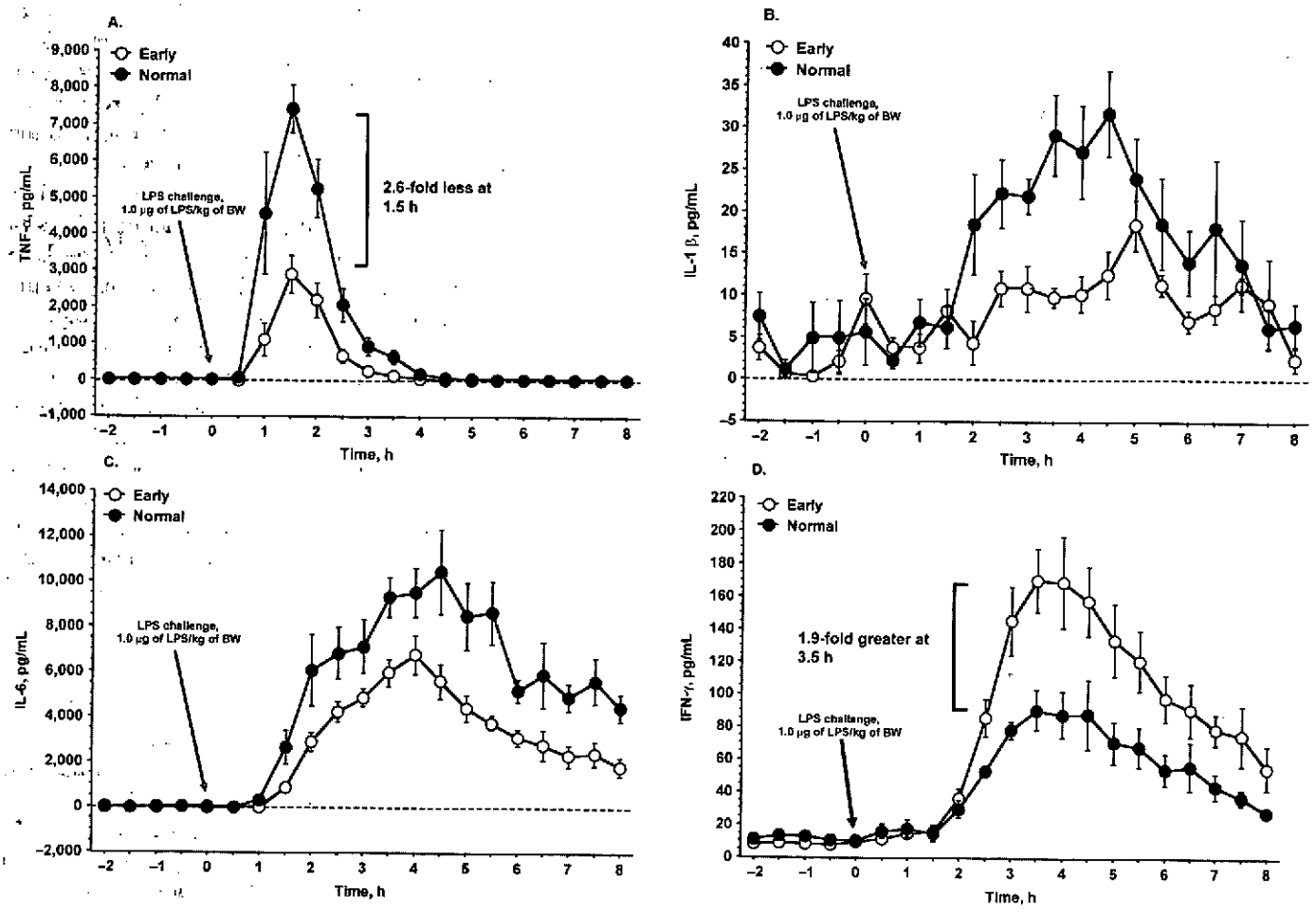

Figure 2. Serum concentrations of tumor necrosis factor (TNF; A), IL-1 (B), IL-6 (C), and interferon-γ (INF; D) in early weaned (EW; n = 8) and normal-weaned (NW; n = 10) Brahman × Angus calves after an intravenous bolus dose of lipopolysaccharide (LPS; 1.0 µg/kg of BW) via jugular catheter immediately after sample collection at time 0. A) TNF: A weaning treatment × time interaction ($P < 0.0001$) was observed such that serum concentrations of TNF were greater in NW vs. EW calves after LPS challenge. Peak serum concentrations of TNF occurred at 1.5 h post-LPS challenge and were approximately 2.6-fold greater ($P < 0.001$) in NW vs. EW calves. B) IL-1: A weaning treatment × time interaction ($P < 0.0001$) was observed such that serum concentrations of IL-1 were greater in the NW vs. EW calves after LPS challenge. Peak serum concentrations of IL-1 occurred at 4.5 h (32 + 5.1 pg/mL) and 5 h (19 + 3.0 pg/mL) post-LPS challenge in the NW and EW calves, respectively. C) IL-6: A weaning treatment × time interaction ($P < 0.0001$) was observed such that serum concentrations of IL-6 were greater in the NW vs. EW calves after the LPS challenge. Peak serum concentrations of IL-6 occurred at 4.5 h (10,455 + 1,888.3 pg/mL) and 4 h (6,782 + 853.5 pg/mL) post-LPS challenge in the NW and EW calves, respectively. D) IFN: A weaning treatment × time interaction ($P < 0.0001$) was observed such that serum concentrations of IFN were less in NW vs. EW calves after the LPS challenge. Peak serum concentrations of IFN occurred at 3.5 h post-LPS challenge and were approximately 1.9-fold greater ($P < 0.007$) in EW calves compared with NW calves.

($P < 0.0001$). Additionally, whereas serum concentrations of TNF and IL-1 did not differ ($P > 0.20$) at 8 h post-LPS between the 2 weaning groups, serum concentration of IL-6 remained elevated ($P < 0.01$) in the NW group compared with the EW group at 8 h post-LPS administration. Serum profiles of IFN were opposite of those observed for the proinflammatory cytokines (TNF, IL-1, and IL-6) in that a weaning treatment × time interaction ($P < 0.0001$) was observed such that serum concentrations of IFN were less in the NW calves compared with EW calves after the LPS challenge (Figure 2D). Additionally, peak serum concentrations of IFN occurred after peak concentrations of IL-1 and IL-6, occurring at 3.5 h post-LPS challenge. Peak concentrations of IFN were approximately 1.9-fold greater ($P < 0.007$) in EW compared with NW calves. By 8 h post-LPS administration, serum concentrations of IFN were still elevated ($P < 0.0001$) above prechallenge concentrations in both weaning groups and tended ($P < 0.08$) to be greater in the EW group compared with the NW group.

Serum APP

On d 0 EW calves tended ($P = 0.07$) to have less plasma ceruloplasmin compared with NW calves (16.0 and 18.8 mg/dL; SEM = 1.15). This pattern of difference between weaning ages continued throughout the sampling period with significance ($P < 0.05$) reported only at time 0 (immediately before LPS infusion; Figure 3). A weaning age × time interaction was observed for plasma ceruloplasmin concentrations ($P = 0.04$), primarily due to the timing of the endotoxin-induced reduction ($P < 0.001$) in plasma ceruloplasmin concentrations (11.6 and 9.2% reduction in NW and EW calves, respectively). Plasma ceruloplasmin concentra-

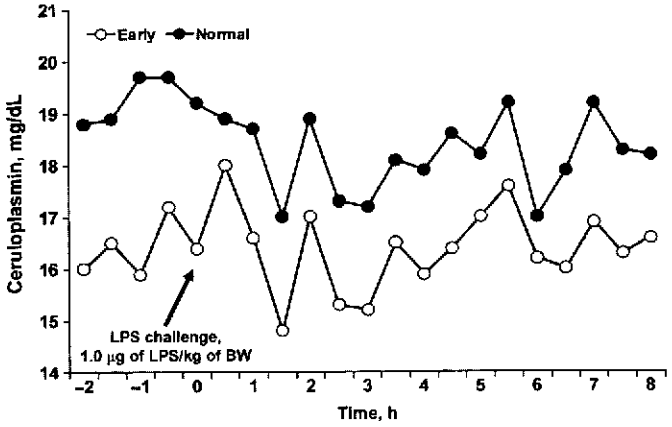

Figure 3. Serum concentrations of ceruloplasmin in early weaned (EW; n = 8) and normal-weaned (NW; n = 10) Brahman × Angus calves after an intravenous bolus dose of lipopolysaccharide (LPS; 1.0 µg/kg of BW) via jugular catheter immediately after sample collection at time 0. A weaning age × time interaction was observed for plasma ceruloplasmin concentrations ($P = 0.04$), primarily because of the timing of the endotoxin-induced reduction ($P < 0.001$) in plasma ceruloplasmin concentrations (11.6 and 9.2% reduction in NW and EW calves, respectively). Pooled SEM = 1.55.

tions in NW calves declined over the 3 sampling intervals after LPS infusion, reaching a nadir at h 1.5. In contrast, EW calves experienced an increase ($P < 0.01$) in plasma ceruloplasmin concentrations in the first post-LPS infusion sampling (0.5 h), followed by a marked decrease ($P < 0.001$) in ceruloplasmin concentrations in the 1.0 and 1.5 h post-LPS infusion samples (Figure 3).

There was no weaning age × time interaction for plasma haptoglobin concentrations ($P = 0.99$); however, EW calves maintained less ($P = 0.03$) haptoglobin concentrations compared with NW calves throughout the 10-h sampling period, irrespective of sampling time (6.9 vs. 7.3 absorption × 100 at 450 nm; SEM = 0.12).

DISCUSSION

In previous studies, EW calves were shown to have a reduced APP response to the stressors associated with weaning, transportation, and feedlot entry (Arthington et al., 2005, 2008). The EW calves in those studies also experienced improved feedlot performance during the initial 30-d receiving period compared with their NW cohorts, despite no differences in morbidity. One possible explanation relates to differences in energy expenditures required for the production of increased inflammatory products, such as the APP. In this manner, activation of the acute-phase reaction may be acting as a nutrient sink, diverting energy and nutrients away from BW gain and toward the production of inflammatory products (Johnson, 1997). Other investigators have reported on the impacts of immune system activation on altered animal growth and feed efficiency (Klasing and Korver, 1997) and nutrient metabolism (Thelen et al., 2007). It was the objective of the current study to examine the influence of immune challenge, through i.v. infusion of LPS, on the activation of the proinflammatory response in calves weaned at an early vs. traditional age. To our knowledge, these data are the first to compare proinflammatory cytokine profiles in EW vs. NW calves, which may assist in the explanation for why EW calves have more efficient BW gain compared with NW cohorts.

Increased circulating cortisol concentrations are a hallmark measure of stress in mammals. In the current study, the postchallenge rise in serum cortisol was expected and was similar to responses previously reported in calves receiving a similar bolus i.v. infusion of LPS (Carroll et al., 2009). The sexual dimorphism, related to greater postchallenge peak cortisol values for heifers vs. steers, has also been reported previously (Arthington et al., 2003) and may simply be due to a general tendency for plasma cortisol to be greater in female vs. male cattle (Henricks et al., 1984).

Each of the 3 primary inflammation-associated cytokines (Gabay and Kushner, 1999) increased in response to LPS-challenge, and in each example concentrations were greater in NW vs. EW calves. These cytokines participate in multiple physiological functions with a primary outcome focused on orchestrating early innate immune responses aimed toward infection-resistance of the host (Suffredini et al., 1999). Although these proinflammatory cytokines participate in important host-protective functions, their actions also result in decreased voluntary food intake and increased tissue degradation, both of which are contrary to efficient food animal growth and development (Johnson, 1997). Similar to our results, other researchers have also reported increased concentrations of proinflammatory cytokines, as a result of endotoxin challenge, in cattle (Kahl and Elsasser, 2006), buffalo (Horadagoda et al., 2002), and reindeer (Orro et al., 2004).

In the current study, an increase in serum ceruloplasmin and haptoglobin concentrations, after LPS challenge, was not observed within the time range of sampling. This was unexpected because these APP are responsive to proinflammatory cytokine regulation, particularly IL-6 (Gabay and Kushner, 1999). The most likely explanation is the timeframe of sampling, where the expected rise in APP concentrations may appear later than 8 h after the inflammatory challenge (Gabay and Kushner, 1999). Nevertheless, mean concentrations of both haptoglobin and ceruloplasmin were greater in NW vs. EW calves, similar to our previous findings (Arthington et al., 2005, 2008). One interesting response of the current study relates to the decline in serum ceruloplasmin concentrations after the LPS challenge. A likely explanation for this response is associated with an adaptive protection response related to iron redistribution in the host. Iron is a limiting nutrient for bacterial growth, and during bacterial infection mammals undergo Fe redistribution, resulting in a decline in Fe from the general circulation in attempt to deprive bacteria of this essential nutrient (Weinberg, 1984). Ceruloplasmin has been shown to be important

for the utilization of Fe by the host (Harris, 1997) via the oxidation of Fe(II) to Fe(III), which is necessary for Fe to bind to transferrin (Osaki and Johnson, 1969).

Although the proinflammatory cytokines are essential for eliciting the APP response, INF has been shown to directly inhibit the IL-6-induced increase in haptoglobin production from bovine hepatocytes (Yoshioka et al., 2002). This evidence supports the results in the current study, where EW calves had greater INF concentrations after LPS challenge, but reduced mean serum haptoglobin concentrations.

Results of the current study reveal that the age of calf weaning significantly influences the release of proinflammatory cytokines (TNF, IL-1, and IL-6) into the blood after i.v. LPS challenge. Based upon these data, the innate immune system of EW calves appears to be less naïve than that of NW calves. Additionally, the differential IFN response indicates that the immune system of EW calves may be more effective at recognizing and eliminating endotoxin. Collectively, these data suggest that an altered innate immune system may be responsible for the improved feedlot performance and reduced APP response previously reported in EW calves entering a feedlot.

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